Determination of Hexazinone in Environmental Samples by Uv-Vis Spectrophotometry-Partial Least Squares Regression

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Abstract

A simple and reliable analytical method is proposed for the spectrophotometric determination of hexazinone in water and soil leachates, where spectral interference from organic matter is avoided by means of a chemometric tool. Binary mixtures containing from 0.5 to 14 µg mL^{-1} of the herbicide and from 0 to 30 µg mL^{-1} of sodium salt of humic acids were prepared for the calibration set. The limit of detection was of 0.1 and the limit of quantification of 0.4 µg mL^{-1}; a precision of 2.0 % was estimated, expressed as a relative standard deviation in percentage. Satisfactory mean recoveries ± confidence limits were obtained in synthetic mixtures (102 ± 2 %), as well as in tap water (102 ± 1 %), well water (103.8 ± 0.3 %) and soil leachates (96 ± 5 %). Unfortunately, a smaller mean recovery was found for sea water (66 ± 15 %), probably due to the high salinity of the matrix reduces the solubility of hexazinone.

Key words: Hexazinone, water, soil leachates, spectrophotometry, PLS, triazines.

Introduction

Since the first triazine was synthesized in 1952, this type of herbicides has been widely adopted in more than 100 countries around the world. Nowadays, two main subgroups are identified: symmetric triazines (e.g. ametryn, atrazine, simazine) and triazinones (e.g. metribuzin, metamitron, hexazinone). The main reasons for their adoption in key crops such as corn, sugarcane, sorghum or forestry are their application flexibility (pre- and post-emergence) and ability to mix with other herbicides for broad-spectrum weed control[1].

Hexazinone, or 3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione according to the IUPAC, is a pre- and post-emergence herbicide used to control many annual grasses and broadleaf weeds in non-cropped lands, as well as crops like alfalfa, blueberries, coffee or sugarcane.

This herbicide may be a cause for concern with regard to environmental contamination due to it is the most water-soluble triazine (33 g Kg[-1]), with a log Koc of 1.30-1.43. Neither hydrolysis nor significant photo-degradation has been observed under normal environmental conditions. Major routes of dissipation are biodegradation and leaching[2,3]. The herbicide shows a half-life of 232 days in anaerobic conditions, while in aerobic conditions it is of 222 days in sandy loam; a half-life dissipation in field has been estimated around 139 days[4]. For analytical purposes, liquid and gas chromatographies[5,6], as well as capillary electrophoresis[7], have been proposed for the quantification of this triazine and other pesticides in water and soil. Also, some authors have proposed its determination in water by derivative UV-Vis spectrophotometric methods[8,9]. For the analysis of technical and formulated pesticides, CIPAC proposes the quantification of hexazinone by liquid chromatography on a C_8 column, using water-acetonitrile 50:50 (v/v) as eluent and UV detection at 254 nm[10].

In relation to Partial Least Squares Type I (PLS-1), it is a powerful regression tool for multi-component analysis when it is used with techniques such as UV-Vis spectrophotometry[11,12], spectrofluorimetry[13] and near and mid-infrared spectrometry[14,15], among others. As it is based on Principal Component Analysis (PCA), the number of original independent variables is reduced to a few new significant variables named Principal Components (PCs) or factors[16,17]. Thus, irrelevant information (i.e. spectral interferences or noise) can be eliminated, enabling to construct a reliable calibration model.

In this work, a novel and simple method for the quantification of hexazinone in water and soil leachates was achieved, based on the multivariant technique of PLS-1 for interferences elimination.

Material and Methods

Instrumentation: A UV-Visible spectrophotometer (Perkin-Elmer, model lambda EZ 210) was used, controlled by a PC while applying the program PESSW v1.2.E by Perkin-Elmer. Data treatment was carried out with the software packages

Pirouette v3.11 by Infometrix Inc., and OriginPro 8 SR0 v8.0724 by OriginLab Corporate.

Reagents and solutions: All the reagents used were analytical grade. Hexazinone (HEXA) was pestanal grade from Riedel-de Häen; sodium salt of humic acids (NaHu) was from Aldrich. Water purified with EasyPure equipment (Barnstead) was used throughout. Stock solutions of HEXA containing 100 µg mL^{-1} and NaHu containing 500 µg mL^{-1} were prepared in water and stored at 4°C. The working solutions were prepared daily through adequate dilution. A buffer solution of K_2HPO_4/KH_2PO_4 0.5 mol L^{-1} pH 8.0 was also used.

Procedure: A training set of 21 samples was prepared for the multicomponent analysis by PLS-1 (see figure 1). Adequate volumes of the stock solutions to obtain final concentrations of HEXA in the range from 0.5 to 14 µg mL^{-1} and from 0 to 30 µg mL^{-1} of NaHu were added to volumetric flasks of 10 mL, in addition to 1 mL of the buffer solution; then the flasks were filled up with water. The absorption spectra were recorded in the range from 200 to 340 nm against a reagent blank, with a spectral resolution of 0.2 nm. The calibration models obtained were validated by using an independent set of 12 test samples with a random composition, which concentrations of HEXA and NaHu were within the ranges considered in the calibration step.

Real water samples were filtered through a nylon membrane of 0.2 µm pore size and fortified with HEXA to estimate the mean recovery. To obtain each soil leachate, 30 mL of water were added to 5 g of soil and maintained in an ultrasound bath for 90 min at ambient temperature; the extract was centrifuged at 5000 rpm during 30 min and the supernatant was mixed with concentrated hydrochloric acid to obtain a final pH of 2. After one hour, the sample was filtered through a nylon membrane of 0.2 µm pore size, neutralized with NaOH and fortified with HEXA; finally, it was analysed in the same conditions as the standard solutions.

Results and Discussion

The absorption spectra of HEXA alone and in presence of NaHu are presented in figure-1, in which a significant spectral interference of NaHu was observed. Therefore, it was considered to use PLS to improve the determination of the herbicide in the presence of organic matter in water and soil leachates.

First, the influence of pH on the spectral characteristics of HEXA was studied. An isosbestic point at 235 nm was observed as a consequence of an acid-base equilibrium. The spectrophotometric determination of pKa[18] was carried out and a value of 1.1 was found. As pH increased, it was detected a hyperchromic effect at the absorption band with a maximum at 245 nm, which correspond to the neutral molecule of HEXA. Moreover, in previous works it was

observed that the soils used to obtain the leachates contained significant quantities of Fe^{3+}, which could be precipitated in basic conditions to eliminate its interference in the spectrophotometric determination. Therefore, a pH of 8.0 was selected. Three buffer solutions were studied: TRIZMA/H_3O^+, $H_2PO_4^-$/HPO_4^{2-}, and $Na_2B_4O_7$/H_3O^+. The phosphate buffer solution did not interfere in the spectral region of work; so it was chosen for subsequent experiments. Concentration of the buffer solution did not show an influence on the absorption spectrum; a concentration of 0.5 mol L^{-1} was selected.
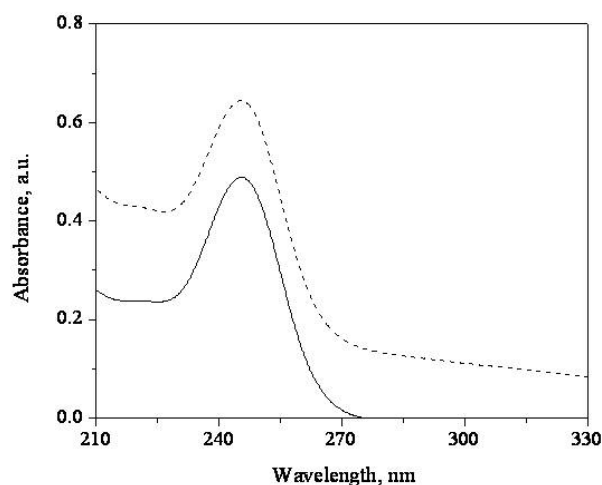

Figure-1
Absorption spectra in water of HEXA (7 µg mL^{-1}) and HEXA with NaHu (7 and 6 µg mL^{-1}, respectively)

Calibration and internal validation: The samples considered for calibration in PLS with HEXA as analyte and NaHu as interference are represented in table-1. HEXA varied in the working concentration range from 0.5 to 14 µg mL^{-1}, previously determined. Samples containing high concentrations of both components were omitted because of their excessive absorbance (>1.2 AU as a maximum). With this arrangement, it was possible to integrate samples with analyte and interferences in different relations, where collinear compositions have shown good predictive capabilities of the calibration model[11,12,19]. A set of twelve independent samples was used for validation[17], in which concentrations of HEXA and NaHu varied in the same ranges as the calibration samples.

The working conditions for PLS were: a) mean centering as data pre-processing strategy[20], b) cross-validation leaving out one sample in each iteration, and c) ten factors as maximum for modeling. One of the most important aspects when proposing a calibration model is the spectral interval. In this case, three models for the calibration step were evaluated: a) model I, from 200 to 340 nm, b) model II, from 205 to 270 nm, where both analyte and interferences absorb radiation, and c) model III, from 220 to 260 nm, a spectral region centered about the absorption band of the HEXA. These

three models were evaluated with regard to their prediction capacity, where both calibration and validation samples were taken into account.

Table-1
Training set of samples used for calibration with PLS-1 (composition in µg mL^{-1})

Sample	HEXA	NaHu
1	0.5	0
2	0.5	6
3	0.5	12
4	0.5	18
5	0.5	24
6	0.5	30
7	1	0
8	1	6
9	1	12
10	1	18
11	1	24
12	4	0
13	4	6
14	4	12
15	4	18
16	7	0
17	7	6
18	7	12
19	10	0
20	10	6
21	14	0

To define the optimal number of factors required to construct the calibration models, PRESS (Prediction Error Sum of Squares) was drawn in function of the ten factors to be evaluated. The results are shown in figure-2. The election of the optimal number of factors was carried out by applying the local minimum criterion with respect to PRESS and the percentage of the accumulated variance[21, 22], where two factors were chosen as optimum.
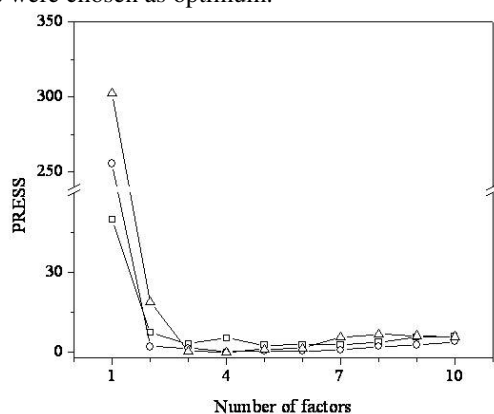

Figure-2
PRESS obtained by cross-validation *vs.* number of PCs or factors used to construct the PLS-1 models proposed: (□) model I, (○) model II, and (△) model III

Then, the statistical parameters of PRESS, RMSD (Root Mean Square Difference), SEC (Standard Error of Calibration), REP % (Relative Error of Prediction in Percentage) and R^2 (Square of Correlation Coefficient) were used to compare the prediction capacity of the calibration models[17]. According to the results shown in table 2, model II gave the best prediction capacity, with the lowest error rate for prediction.

Table-2
Cross-validation of the proposed models based on PLS-1, using the calibration set of samples. Two factors were used in all cases

Parameter	Model I	Model II	Model III
Spectral range	200 - 400	205 - 270	220 - 260
Number of independent variables	701	326	201
Cumulative variance, %	92.5	99.2	99.7
PRESS	7.5	0.4	18.8
RMSD	0.6	0.1	0.9
SEC	0.6	0.1	1.0
REP, %	16.0	4.0	24.0
R^2	0.996	0.999	0.970

External validation: Afterwards, the calibration models proposed by PLS-1 were applied to estimate the concentration of HEXA in synthetic mixtures (validation set). The average percentages for recovery with their confidence limits (R ± LC) as well as the RMSD, SEP (Standard Error of Prediction), the REP (%), and R^2 were calculated for these samples, of which the results are indicated in table 3. As can be seen, the best results were obtained with model II, which coincides with those obtained by cross-validation (table 2). Due to the estimated parameters with model II in the cross and external validation are in the same magnitude range, neither over- nor under-fitting effects were observed, which confirms that two factors were adequate to construct the calibration model. Therefore, model II was chosen for subsequent studies with a satisfactory prediction in samples where the relation HEXA:NaHu is up to 1:60.

Table-3
External validation of the proposed models based on PLS-1, using the validation set of samples. Two factors were used in all cases

Parameter	Model I	Model II	Model III
R ± CL[a]	83 ± 2	102 ± 2	106 ± 2
PRESS	34.7	0.2	1.9
RMSD	1.3	0.1	0.3
SEP	1.3	0.1	0.3
REP, %	15.8	1.3	3.7
R^2	1.2	1.0	1.0

[a] Mean recovery in percentage with their confidence limits (α = 0.05, eleven degrees of freedom, two-tailed t-student test).

Figure 3 represents the correlation between the expected and the estimated concentration of HEXA for the calibration and validation series. In the case of the validation series, the relationship $C_{predicted}$ *vs* $C_{expected}$ corresponds to a correlation coefficient of r = 0.9998. The repeatability of the method was 2.0 % expressed as a relative standard deviation in percentage, based on a series of 10 samples with 7 µg mL^{-1} of HEXA and 7 µg mL^{-1} of NaHu. A limit of detection of 0.13 µg mL^{-1} and a limit of quantification of 0.43 µg mL^{-1} were estimated[23].

Water samples and soil leachates: To evaluate the capacity of the proposed calibration model to quantify HEXA,

samples of tap, well, and sea water were fortified with the herbicide as shown in table-4. The analyses were carried out in triplicate (except for tap water), of which the results are reported in the same table. As can be seen, the greatest errors in the determination of HEXA were found at low concentrations in water from well II (near a local beach) and from sea water; it is probable that the high salinity of the matrix reduces the solubility of HEXA in these samples. On the other hand, the determination of HEXA in drinking and well I water was satisfactory in the interval of the studied concentrations.
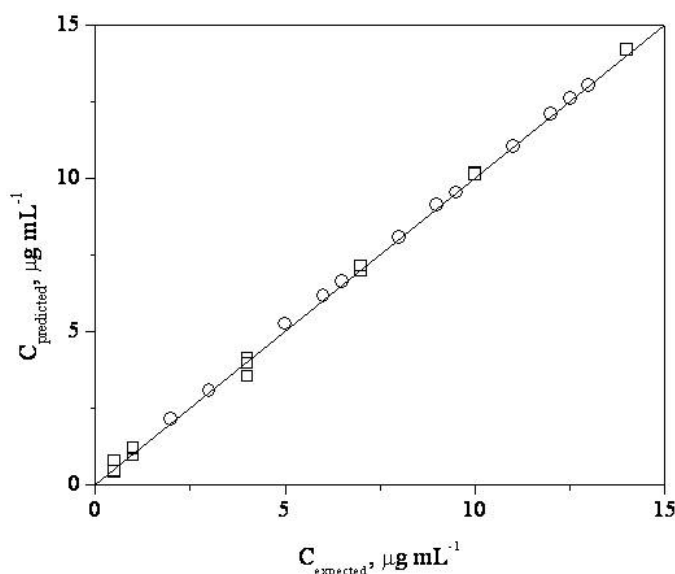

Figure-3
$C_{predicted}$ *vs* $C_{expected}$ of HEXA in (□) calibration and (○) validation sets of samples

Table-4
Analyses of real water samples fortified with HEXA. All concentrations are in µg mL^{-1}

Actual concentration	Found concentration			
	Tap water[a]	Well I water	Well II water	Sea water
2	2.0	2.07 ± 0.03	0.9 ± 0.3	0.30 ± 0.04
5	5.2	5.19 ± 0.08	4.0 ± 0.1	3.5 ± 0.1
7	7.2	7.27 ± 0.08	6.1 ± 0.2	5.5 ± 0.2
9	9.2	9.4 ± 0.1	8.2 ± 0.2	7.4 ± 0.5
12	12.4	12.48 ± 0.03	11.3 ± 0.4	10.3 ± 0.3
R ± CL[b]	102 ± 1	103.8 ± 0.3	80 ± 10 (88 ± 4)[c]	66 ± 15 (79 ± 4)[c]

[a] Samples analysed without replicates. [b] Mean recovery in percentage with their confidence limits (α = 0.05, two degrees of freedom, two-tailed t-student test). [c] Same parameter, with the elimination of sample fortified with 2 µg mL^{-1}.

Also, two kinds of soil with different characteristics were used to obtain the leachates fortified with HEXA (table-5); organic carbon in leachates is associated with the presence of humic matter[24]. The obtained results were satisfactory in both cases. Thus, the method developed for the quantification of HEXA was satisfactory in terms of exactitude and precision, even when analysing aqueous samples with a high content of organic matter.

Table-5
Soil characteristics and analyses of corresponding aqueous leachates fortified with HEXA

Property	Soil I	Soil II
Texture	Sandy loam	Clay loam
Organic matter, in percentage	1.2	1.0
Organic carbon, in percentage	0.7	0.6
Organic carbon estimed in leachates, $\mu g\ mL^{-1}$	41	30
Quantification of HEXA, $\mu g\ mL^{-1}$		
Actual concentration	Leachate I	Leachate II
2	1.6 ± 0.3	1.6 ± 0.1
5	4.7 ± 0.6	4.6 ± 0.2
7	7.1 ± 0.1	6.9 ± 0.1
9	9.0 ± 0.1	9.1 ± 0.1
12	12.6 ± 0.8	12.2 ± 0.1
R ± CL[a]	96 ± 5	96 ± 4

[a] Mean recovery in percentage with their confidence limits (α = 0.05, two degrees of freedom, two-tailed t-student test)

Conclusions

A fast and reliable method is proposed for the determination of HEXA in tap and well water, such as aqueous soil leachates. Organic matter interferences were avoided by means of multivariate calibration using the PLS-1 strategy, in which a binary matrix was designed with HEXA as analyte and sodium salts of humic acids as interferences. Satisfactory results were obtained during internal and external calibration, for the calibration model with the spectral range from 205 to 270 nm, mean centering data, and two factors for modeling. Unfortunately, the results obtained for sea water were not satisfactory; probably the high salinity of the matrix reduces the solubility of the herbicide.

Acknowledgement

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